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SYMPOSIUM: Peripheral Neuropathies

# Neurotrophic Factors in Peripheral Neuropathies: Therapeutic Implications

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**Neurotrophic factors are proteins which promote the survival of specific neuronal populations. Many have other physiological effects on neurons such as inducing morphological differentiation, enhancing nerve regeneration, stimulating neurotransmitter expression, and otherwise altering the physiological characteristics of neurons. These properties suggest that neurotrophic factors are highly promising as potential therapeutic agents for neurological disease.**

**Neurotrophic factors will most likely be applied to the peripheral nervous system initially, since there are fewer problems for large proteins to gain access to peripheral neurons. Many of the most intensively studied factors are active in the peripheral nervous system. These include the neurotrophins (nerve growth factor, brain derived neurotrophic factor, neurotrophin-3, neurotrophin-4/5), the insulin like growth factors, ciliary neurotrophic factor, and glial cell derived neurotrophic factor and its related proteins. The biology of these factors and their receptors in the peripheral nervous system is reviewed here. We also review data suggesting that abnormal availability of some factors may contribute towards the pathogenesis of certain types of peripheral neuropathy. Finally, the pre-clinical data suggesting that individual factors might be effective in treating neuropathy is reviewed, along with data relating to possible side effects of neurotrophic factor therapy. Several factors have already entered clinical trials with variable success. The data from these trials is reviewed as well**

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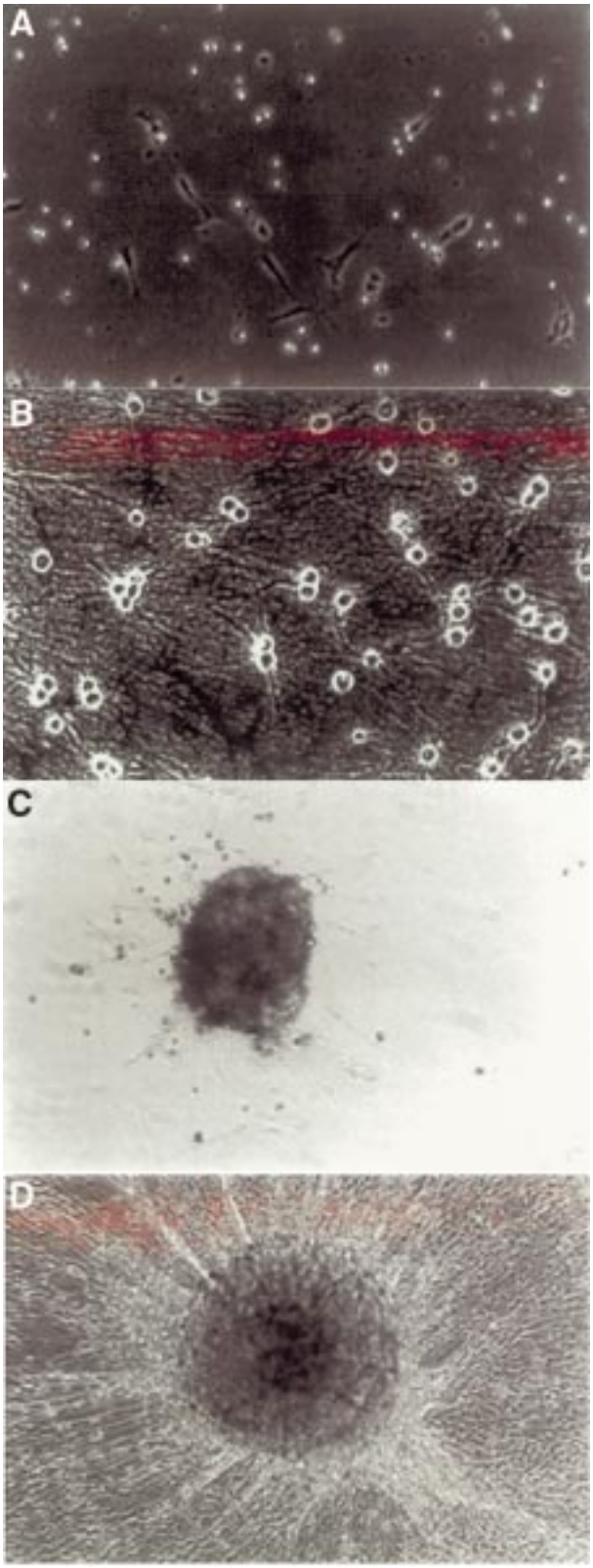
## Introduction

Conventional treatments for peripheral neuropathy have primarily been palliative rather than curative, and symptom oriented rather than disease oriented. Perhaps most importantly, they have often been ineffective. In recent years enormous progress has been made in our understanding of the biology of neurotrophic factors and how they may be applied to neurologic disease. These advances have suggested new therapeutic approaches that may arrest or reverse the disease process underlying many types of peripheral neuropathies. Some of these approaches promise to be uniquely effective in treating what is often a relentlessly progressive degenerative disease. Several different neurotrophic factors have already been tested in clinical trials for the treatment of peripheral nerve disease, with variable results. Others are being considered for clinical trials in the near future. This manuscript will briefly review some of the relevant biology of these factors, their role in the peripheral nervous system, and their potential role as therapeutic agents for peripheral neuropathy.

## What are neurotrophic factors?

The word "trophic" is derived form the Greek word *trophikos*, which means "nourishment." The word "neurotrophic" can therefore be translated as "neuron nourishing." In modern usage, the term neurotrophic is applied to substances (generally proteins) which promote the survival of neurons. This is to be distinguished from a very similar sounding word, "neurotropic," which is derived from the Greek word "*tropikos*," meaning "causes to turn." The term "neurotropic" is applied to substances which influence the direction of neurite outgrowth during development or regeneration. The similar sounding words are potentially the source of much confusion, but fortunately many substances that are "trophic" are also "tropic," obviating the need to distinguish between these two properties in many circumstances. For the purposes of this discussion, we will define neurotrophic factors as proteins which promote




the survival of neuronal populations (Figure 1). Keep in mind, however, that many such factors also stimulate neurite outgrowth, promote regeneration, influence neurotransmitter expression, and otherwise regulate gene expression in neurons. Many also have a variety of effects on non-neuronal cells. All of these properties will need to be considered when evaluating the potential usefulness of these factors in the clinical setting.

**Neurotrophins**

The first neurotrophic factor to be discovered was nerve growth factor (NGF). This discovery came about in the early 1950's as a result of the observation that sensory ganglia became hypertrophied when mouse sarcoma tissue was grafted onto a chick embryo in place of an extirpated wing bud (98). Subsequent studies identified the responsible factor as a protein consisting of two linked identical peptide chains, each containing 118 amino acid residues, and integrated into a larger protein complex called 7S NGF after it's molecular size (32). The fortuitous discovery that NGF was present in relatively large quantities in mouse salivary glands facilitated investigative work with this newly discovered factor. In fact, although other neurotrophic factors were subsequently discovered over the next few decades, virtually everything known about them, until relatively recently, was derived from studies of NGF.

Antibody studies demonstrated that NGF was a naturally occurring protein that appeared to play an important role in the normal development of peripheral sensory and sympathetic ganglia (32). NGF-antiserum administered *in vivo* caused sympathetic and sensory neurons to die during development. The essential role of NGF in the development of these peripheral populations was confirmed in more recent studies when transgenic mice with a null mutation of the NGF gene were born with virtually no sympathetic neurons and a more than 70% reduction in the normal number of dorsal root ganglion sensory neurons (38). It was discovered that NGF is released from target tissue during development, bound to specific receptors, and retrogradely transported back

**Figure 1.** Trophic and tropic effects of NGF. Figures A and B show neonatal sympathetic neurons grown in dissociated tissue culture. **A**) When no NGF is added to the medium, virtually all the neurons die. The only cells seen are non-neuronal. **B**) When the medium is supplemented with NGF most neurons survive and extend neurites. Figures C and D show dorsal root ganglia grown in explant culture. **C**) With no NGF added only a few small neurites grow. **D**) When NGF is added to the medium large healthy processes surround the ganglion.

to the cell body where it exerts its effects on gene expression (70). During development many neurons grow towards their intended targets in numbers that are way in excess of what would be needed to establish connections in the mature nervous system (125). Embryonic neurons compete for limited quantities of neurotrophic factors released by the target tissues. Those neurons unable to get adequate trophic support die, those which could survive and establish synaptic connections (41, 99). These observations led to the formulation of a broad neurotrophic hypothesis, which states that neuronal survival depends on the availability of target derived neurotrophic factors (Figure 2).

Years after the discovery of NGF, neurotrophic activity was discovered in glial conditioned medium which was not inhibited by NGF antiserum. This activity was purified from pigs brain and called brain derived neurotrophic factor (17). It differed from NGF in that BDNF clearly supported different populations of neurons (see Table 1). Structural analysis, however, revealed that there was greater than a 50% homology in amino acid sequence between the two proteins, suggesting that they were members of a common gene family (97). The high degree of structural similarity initiated a search for other members of this gene family resulting in the nearly simultaneous discovery of neurotrophin - 3 (NT-3) by six different groups of investigators (50, 72, 84, 85, 111, 134). Subsequently, other members of the neurotrophin family were discovered. NT-4 was isolated from Xenopus and Viper (61), and NT-5 from human placenta (20). It soon became apparent, however, that these two factors are likely to be inter-species variants of the same protein, and are commonly referred to as NT-4/5. More recently a new protein was discovered in fish with structural similarity to the other neurotrophins (59). NT-6 differs from the other neurotrophins, however, in that it is membrane bound and requires activation with heparin.

The neurotrophin gene family mediate their actions by binding to a corresponding gene family of receptor tyrosine kinases called trks (tropomyosin related kinase). The original trk protein (also referred to as trk A) was identified as the proto-oncogene associated with an oncogene isolated from colon carcinoma cells (112). It was subsequently discovered that trk bound NGF with high affinity, was expressed on NGF responsive cells, and was able to mediate the biological effects of NGF when bound with receptor dimerization (81, 86, 88). Other members of the trk gene family were found to bind the other neurotrophins with high affinity and mediate their biological effects. Trk B serves as a receptor for both BDNF and NT-4/5, while trk C serves as the receptor for NT-3 (89, 90, 94). NT-3 is also capable of binding to trk A and trk B. Although it binds these receptors with high affinity, NT-3 is much less efficient than the other neurotrophins in eliciting biological responses through trk A and trk B. This story is further complicated by the fact that both trk B and trk C may be expressed as truncated receptors which lack intracellular catalytic domains (87, 154). The function of these truncated forms of the trk receptors is currently not understood, but they are highly abundant in both the central and peripheral nervous systems.

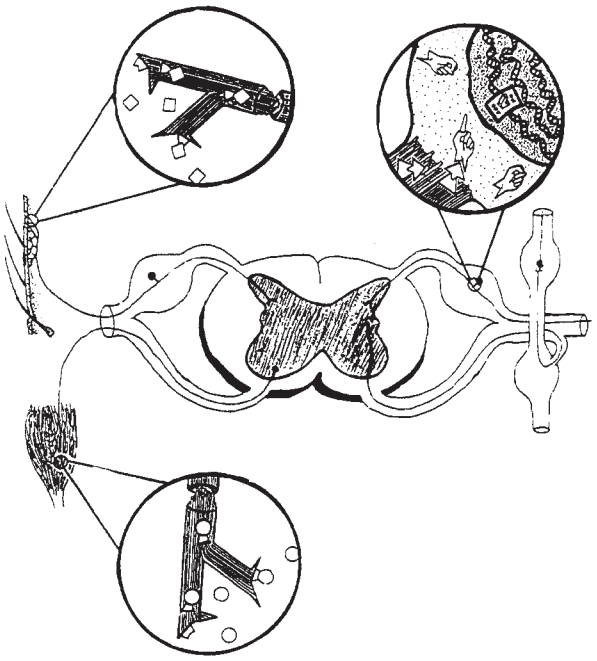


**Figure 2.** Neurotrophic hypothesis. Developing neurons compete for neurotrophic factors released by target tissues in the periphery. Once released, the neurotrophic factors bind to specific receptors on responsive neurons, are internalized and retrogradely transported back to the cell body where they exert their effects by second messenger signalling which in turn regulates gene expression in the nucleus.

| NGF | BDNF<br>NT-4/5 | NT-3 |
|---|---|---|
| Neural crest derived - small fiber sensory | Neural crest derived - medium fiber sensory | Neural crest derived - large fiber sensory |
| Sympathetic | Placode derived sensory | Placode derived sensory |
| | Motor | Sympathetic |
| | | Motor |

**Table 1.** Peripheral populations responsive to the neurotrophins.

In addition to the trk high affinity receptors, another receptor which binds all the neurotrophins with low affinity had been identified. This receptor, called p75 because of its 75 kD molecular weight, does not signal with tyrosine kinase activity (83). Although it may enhance the affinity of trk A for NGF (67), p75 also appears to mediate some of the biological effects of NGF directly. For example, NGF binding to p75 induces melanoma cells to invade through basement membrane (71), and can promote Schwann cell migration (6). Transgenic mice with a null mutation for p75 have reduced sensory innervation by fibers containing substance P and calcitonin gene related peptide (CGRP), both of which are abundant in NGF responsive sensory neurons (95). In these same animals, sensory neurons and sympathetic neurons display reduced sensitivity to NGF during development (96). In addition to potentiating the response of trk to NGF binding, p75 may also directly mediate the NGF signal through activation of a sphingomyelin based pathway (44) and subsequent activation of NF-κB (27). The p75 receptor may also play an important role in mediating apoptosis through its association with NGF (28, 55). Although p75 has not been shown to mediate the biological effects of the other neurotrophins, it may play a role in facilitating the retrograde transport of BDNF and NT-4/5 (40).

### Role of Neurotrophins in the Peripheral Nervous System

Almost from the time that NGF was first discovered, it became clear that the neurotrophins played a major role in the peripheral nervous system. It was observed at that time that NGF promoted the survival of nearly all post ganglionic sympathetic neurons, some sensory neurons but no motor neurons or parasympathetic neurons (98,99). The role of NGF in the sensory ganglia seemed to be complex. It was apparent that only a subpopulation of neural crest derived sensory neurons responded to NGF and that no placode derived sensory neurons did. Numerous *in vitro* and *in vivo* studies have confirmed that the different neurotrophins are selective for different subpopulations of sensory neurons (see Table 1). NGF supports the survival primarily of neural crest derived, small fiber sensory neurons that mediate pain and temperature sensation (58, 153). BDNF and NT-4/5 support populations of placode derived sensory neurons as well as neural crest derived neurons which range from small to large diameter and may convey mechanical pressure sensation (51, 105). NT-3 is trophic for the largest diameter fiber neural crest derived sensory neurons which convey proprioceptive and vibratory information (52, 73). The actual situation is somewhat more complex, however, since it is now known that expression patterns for the different trk receptors change during development, as does responsiveness to the different neurotrophins (108). In addition, many dorsal root ganglion neurons express more than one type of trk receptor at a time (114). Motor neurons, for example, express both trk B and trk C, and as would be expected they are responsive to BDNF, NT-3 and NT-4/5, although not necessarily with the same potency. Of the neurotrophins, BDNF appears to be the most potent in promoting the survival of motor neurons.

Most of the above discussion relates to developing neurons, it is less certain what role the neurotrophins play in the mature neuron. For example, unlike developing sensory neurons, mature neurons do not require neurotrophin support for survival in tissue culture (106). Why this is so is unknown, however, mature sensory neurons express BDNF themselves, suggesting the possibility of an autocrine role for BDNF (1). Whether they are needed for neuronal survival or not, the neurotrophins do have a variety of effects on adult neurons. NGF stimulates the production of substance P and CGRP in adult sensory neurons (107), induces hyperalgesia in mature animals (100), protects against a variety of nerve injuries (to be reviewed in detail below), and increases the expression of BDNF in mature sensory ganglia (13). NT-4/5 has been shown to attenuate decreases in motor nerve conduction velocities following axotomy, and NT-3 attenuated a loss of conduction velocity in both sensory and motor nerves in the adult rat (122). These observations suggest that the neurotrophins may continue to play a physiological role in the mature peripheral nervous system, and raise the possibility that they may be useful in treating disorders affecting the peripheral nerve.

### Ciliary Neurotrophic Factor (CNTF)

CNTF was initially purified from chick ocular tissues, where it was believed to be the soluble protein which promoted the survival of parasympathetic ciliary ganglion neurons (16). Although it did promote the survival of these neurons, it soon became apparent that CNTF lacked an amino terminal signal sequence and therefore was probably not secreted, making it an unlikely candidate for a target derived growth factor (104). The structure of CNTF was also interesting in that it closely resembled certain hematopoietic cytokines, rather than any of the neurotrophins. In particular CNTF resembled leukemia inhibitory factor (LIF) with which it shared the ability to induce cultured

sympathetic neurons to change from a noradrenergic phenotype to a cholinergic one (136, 160). The receptors for CNTF and LIF share a heterodimer of two proteins called gp130 and LIF receptor-β, and in addition each receptor differs in another component called the α component (42). CNTF-α, which is restricted in expression to the nervous system, is the component of the receptor that actually binds to CNTF and confers its specificity (75). Other cytokines which are related in structure and similarity of their receptors include interleukin-6, oncostatin M and cardiotrophin-1 (18).

In addition to parasympathetic ciliary neurons, CNTF supports subpopulations of sensory neurons and sympathetic neurons (see Table 2). CNTF was the first neurotrophic factor discovered to be capable of promoting the survival of motor neurons *in vitro* (14) and *in vivo* (142). CNTF also promotes sprouting of motor neurons when injected over the surface of the gluteus muscle (60), and hastens the maturation of motor nerve conduction velocity in juvenile rats (9). Whether CNTF plays a physiological role in the development or maintenance of the motor system is unclear. In the peripheral nerve CNTF is expressed only in Schwann cells, and since it lacks a signal sequence is not likely to be a target secreted growth factor. Null mutations of CNTF in mice fail to result in significant loss of motor neurons at birth, although there is a mild loss as the animals age (113). In a human study it was reported that 2.3% of the Japanese population are homozygotic for a null mutation of the CNTF gene, but fail to demonstrate any associated motor neuron disease, or any other neurological disease (150). When null mutations eliminating the expression of the CNTF-α receptor in mice were created, however, the mice displayed severe motor deficits (43). This suggests that perhaps some other CNTF like factor which also binds to the CNTF-α component is essential for normal motor neuron development.

The lack of a signal sequence suggests that CNTF is not normally released. Since it has significant trophic activity on motor neurons, however, it was postulated that perhaps CNTF might serve as an injury related factor which is released when the integrity of a nerve is compromised. In support of this it was discovered that CNTF is found in significant quantities at extracellular sites along the nerve up to seven days following axotomy (143). In addition, retrograde axonal transport of CNTF by motor and sensory neurons is increased following a peripheral nerve lesion (40). In one interesting study, pmn mice who naturally develop progressive degeneration of motor neurons were subjected to facial nerve transection (145). This lesion significantly

| Peripheral Populations Responsive to CNTF |
|---|
| Neural crest derived - sensory |
| Parasympathetic |
| Motor |

**Table 2.**

| Insulin | IGF-I | IGF-II |
|---|---|---|
| Sympathetic | Neural crest - sensory? | Neural crest - sensory |
| Motor | Sympathetic | Sympathetic |
| | Motor | Motor |

**Table 3.** Peripheral populations responsive to insulin and the IGFs.

increased the number of surviving motor neurons in mice that normally express CNTF, but did not in pmn mice lacking CNTF. This suggests that CNTF released from lesioned Schwann cells protects the motor neurons from degeneration.

### Insulin Like Growth Factors

Insulin is a member of a family of structurally and functionally related proteins that includes insulin like growth factors I and II (IGF-I, IGF-II). They have broad somatic activities associated with the regulation of body growth during development. Each of the three family members has its own distinct receptor, but there is considerable cross reactivity so the two IGF receptors are referred to as the type-I and the type-II IGF receptors (124). The IGFs and their receptors are widely expressed in the peripheral nervous system during development and in maturity (102). Their natural function in the peripheral nervous system is not fully understood, but there is evidence suggesting that they promote nerve regeneration. Expression of both IGFs is increased in muscle following denervation, but returns quickly to baseline after regeneration takes place (47). *In vitro*, the IGFs promote neurite outgrowth of sensory, motor and sympathetic neurons (132). *In vivo*, IGF-II has been shown to promote the regeneration of both motor and sensory nerve fibers following sciatic nerve crush (57,76,123). Conversely, antiserum to the IGFs inhibits motor and sensory nerve regeneration.

Whether the IGFs promote neuronal survival or not has been a more difficult question to answer conclusively. *In vivo*, IGF-I prevents developmentally related

| GDNF | Neurturin | Persephin |
|---|---|---|
| Motor | Motor | Motor |
| Neural crest - sensory | Neural crest - sensory | |
| Nodose - sensory | Nodose - sensory | |
| Sympathetic | Sympathetic | |
| Parasympathetic | | |

**Table 4.** Peripheral populations responsive to GDNF family members.

motor neuron death in chick embryos and attenuates motor neuron death following axotomy (102). When tested *in vitro*, however, IGF-I was only able to promote the survival of motor neurons when cultured in the presence of astrocytes (5), suggesting that it may have only an indirect effect. Similarly, IGF-I promotes the survival of parasympathetic ciliary neurons, but only in the presence of a protein kinase C activator (37). IGF-I fails to promote the survival of sensory neurons *in vitro* (102).

### Glial cell line- Derived Neurotrophic Factor (GDNF)

GDNF was originally discovered in the course of a search for the factor in glial cell conditioned medium which promoted the survival of ventral midbrain dopaminergic neurons (49, 103). Once purified, it quickly became apparent that this neurotrophic factor was the most potent factor yet discovered to act on this population of dopaminergic neurons. In addition to promoting survival, GDNF also stimulates their differentiation and high affinity dopamine uptake (149). It is widely expressed throughout the central and peripheral nervous system, as well as in nonneuronal tissues, suggesting a functional role outside the nervous system. Among the most severe defects found in mice carrying null mutations of the GDNF gene was the failure to develop kidneys. GDNF signals through a receptor complex that includes the Ret tyrosine kinase and a member of the GFRα family of glycosylphosphatidylinositol (GPI) linked co-receptors (81, 140). Either GFRα-1 or GFRα-2 can bind GDNF and together with Ret serve as a functional receptor, however, GDNF preferentially binds to GFRa-1.

Early on it was discovered that GDNF is trophic for other populations of neurons in addition to the dopaminergic ones. Most importantly it was found to be a highly potent neurotrophic factor for embryonic motor neurons (68). In fact early reports suggested that it was more potent than either CNTF or the neurotrophins at promoting motor neuron survival *in vitro*. *In vivo*, GDNF prevented the degeneration of facial and spinal motor neurons (126, 161). It is found to be expressed in muscle and ventral spinal roots as well as in Schwann cells, suggesting that it might play a physiological role in promoting motor neuron survival (68). The lack of severe motor neuron defects in GDNF knockout mice, however, suggests that motor neurons are not exclusively dependent on it (121). While GDNF is very effective promoting the survival of motor neurons, it may be much less effective at preventing motor neuron process degeneration. It failed to prolong the survival of pmn mice despite reducing the loss of motor neuron cell bodies (138). The reason became apparent when it was discovered that motor nerve degeneration was not prevented by GDNF administration in this model. These results raise serious questions about the potential usefulness of GDNF as a therapeutic agent for motor neuron degenerative diseases. GDNF is also a potent neurotrophic factor for populations of sensory, sympathetic and parasympathetic neurons (25), suggesting that it plays an important role in the peripheral nervous system, and may prove to be of use apart from its potential for motor neurons (Table 4).

GDNF is structurally a novel member of the transforming growth factor beta (TGF-β) superfamily of growth factors (103). Subsequent to its discovery, other more closely related factors have been discovered which also may prove to be important neurotrophic factors for the peripheral nervous system. Neurturin was discovered as the factor in conditioned medium from Chinese hamster ovary cells which promoted the survival of cultured sympathetic neurons (93). It has about 40% homology to GDNF, and a very similar spectrum of responsive neurons including motor, some sensory, and autonomic neurons in the peripheral nervous system. Considering the overlap in activity, it is not surprising that neurturin shares a receptor signaling complex with GDNF, although neurturin preferentially pairs with GFRα-2 rather than GFRα-1 (15, 91). More recently, a third member of this gene family has been cloned. Persephin also shares about 40% homology with the other two factors, but appears to utilize a different receptor complex for signaling (117). Consequently, persepherin differs from GDNF and neurturin in that it does not promote the survival of peripheral neurons other than motor neurons.

### Are Neurotrophic Factors Involved in the Pathogenesis of Peripheral Neuropathy?

Since neurotrophic factors are naturally occurring proteins which play an important role in the develop-

ment and maintenance of the nervous system, it is reasonable to imagine that dysfunction of these factors or their receptors might play a role in the pathogenesis of some neurological disease. Many of the null mutations that have been induced in transgenic animals have resulted in abnormal nervous system development. Nevertheless, in most instances it is not clear what relationship these abnormalities may have to clinical disease entities.

It would be expected that abnormalities of gene expression would result in congenital disorders. A few congenital disorders affecting the peripheral nervous system have been described which may relate to abnormal neurotrophic factor expression. For example, congenital sensory neuropathy with anhydrosis (hereditary sensory and autonomic neuropathy type IV), an autosomal-recessive disorder characterized by unexplained fevers, anhidrosis, absent reaction to pain, self mutilating behavior and mental retardation, has been found to be associated with mutations to the trk A gene (74). In another example, a single patient has been described with sympathetic dysfunction and loss of tyrosine hydroxylase, neuropeptide Y (sympathetic markers), substance P and CGRP (sensory neuropeptides), with depletion of unmyelinated small diameter fibers on nerve biopsy (3). This patient was found to have abnormally low levels of NGF expression in skin and nerve biopsy sections. Abnormal expression of NGF was also considered to be a possible cause of Riley-Day syndrome (familial dysautonomia), a congenital disease characterized by loss of pain sensation and autonomic dysfunction. Examination of the NGF gene and that of the p75 receptor failed to reveal any abnormalities (22, 23), and NGF was discovered to be secreted normally from fibroblasts harvested from these patients (48). However, NGF recovered from these fibroblasts has been reported to have abnormally low levels of activity (141).

A possible pathogenic role for neurotrophic factors in acquired neuropathies, and in particular diabetic neuropathy, has been extensively investigated. NGF levels were found to be reduced in sympathetic target tissues immediately after the induction of diabetes in streptozocin-treated rats (64). Later levels of NGF were elevated in these tissues, and reduced in sciatic nerve and sympathetic ganglia, suggesting that retrograde transport of the target derived growth factor was impaired. Others have demonstrated more directly that retrograde transport of NGF is impaired (66, 79). NGF levels are also reduced in submandibular glands and serum from diabetic rodents (127), and in serum from human diabetic patients (53). These studies suggest that there might be impaired production of NGF as well as transport in diabetic subjects. In support of this, it has been demonstrated that levels of NGF mRNA are reduced in muscle and skin of the rat hindfoot in proportion to the duration of diabetes (24). Abnormalities of NGF production and retrograde transport may be prevented with improved glucose control (65). In addition to abnormalities of NGF itself, trk A receptor mRNA is also reduced in dorsal root ganglia of diabetic rats (110). None of the above studies establishes a direct connection between abnormal NGF availability and clinical diabetic neuropathy, but a recent study is strongly suggestive. Diabetic subjects were found to have an early dysfunction of small fiber sensory and autonomic fibers as measured by temperature sensitivity and capsaicin-induced axon reflex vasodilation, prior to the appearance of clinical symptoms of neuropathy, and this dysfunction correlated with reduced levels of NGF in the skin (4). This suggests that NGF deprivation may be responsible for early small fiber dysfunction in patients with diabetic neuropathy. Small fiber dysfunction contributes towards many of the symptoms and complications of diabetic neuropathy including skin ulceration (128). Relatively little information is available regarding other neurotrophins in diabetic neuropathy, however, there is one report that levels of NT-3 mRNA are reduced in muscle from diabetic rats (152).

IGF-I levels have been reported to be low in diabetic patients while levels of the IGF-I binding protein-I are elevated (36). Since the binding protein may competitively inhibit the binding of IGF-I to its receptor, this would result in even lower IGF-I activity. Levels of mRNA for both IGF-I and IGF-II are reduced in the sciatic nerve of diabetic rats (159). It has been proposed that decreased levels of insulin in diabetic patients might lead to reduced IGF levels, and that in turn predisposes the nerves to neuropathy (77). No direct correlation has been established, however, between IGF depletion and clinical diabetic neuropathy.

Overall the data suggesting a possible correlation between abnormal neurotrophic factor availability and the cause of any particular neuropathy is intriguing but far from conclusive. The rationale for the use of neurotrophic factors in the treatment of peripheral neuropathy is not dependent on an association with the pathogenesis of the disease. Regardless of whether neurotrophic factor deprivation is a major cause of neuropathy, a minor contributory factor, or unrelated, the primary reason to consider growth factor therapy lies in the general properties of these proteins. They promote

| Therapeutic Agents | | Environmental Toxins |
|---|---|---|
| **Antineoplastic agents:** | Vincristine<br>Cisplatin<br>Paclitaxel | Acrylamide<br>AETT<br>Buckthorn Toxin<br>Cadmium |
| **Antibiotics:** | Nitrofurantoin<br>Chloroamphenicol<br>Flagyl<br>Isoniazid<br>Clioquinol | Carbon Disulfide<br>n-Hexane<br>Methyl-Butylketone<br>Lead<br>Mercury<br>Organophosphates |
| **Anti-viral agents:** | Dideoxycytidine<br>Dideoxyinosine | Arsenic<br>Bromophenylacetylurea<br>Thalium |
| **Cardiac drugs:** | Hydralazine<br>Ergotomines | Methyl bromide<br>Methyl chloride<br>Phenol |
| **Other drugs:** | Acetazolamide<br>Bismuth<br>Colchicine<br>Phenytoin<br>Disulfiram<br>Pyridoxine | |

**Tabel 5.** Agents toxic to the peripheral nervous system.

the survival of particular populations of neurons, making them more resistant to injury. Some factors may also stimulate nerve regeneration, or enhance the normal physiological functions of surviving neurons. These actions may improve the clinical condition of neuropathic patients, regardless of the cause of injury.

### Treatment of Toxic Neuropathy

Many of the original studies which suggested that neurotrophic factors might be useful in the treatment of peripheral neuropathy focused on toxic neuropathies. Toxic neuropathies were a logical place to start for several reasons. First, it was relatively easy to establish animal models which were similar to the clinical disease. Second, the disease entity itself is relatively simple and practical from the point of view of establishing a new type of therapy. The time of exposure is usually known, and in the case of iatrogenic toxic neuropathy it may be anticipated. The time course and clinical progression of the disease is known or may be predicted. In addition, the underlying pathology is usually well defined, including a recognition of the susceptible neuronal populations. Consequently, it is relatively simple to choose the most appropriate neurotrophic factor, and to administer it even before the neuropathy appears. Finally, the toxic neuropathies for the most part primarily injure sensory neurons, and sensory neurons are not protected by the blood nerve barrier, so they have relatively easy access to systemically administered proteins.

Iatrogenic toxic neuropathy in particular is becoming an increasingly important clinical problem. Many new therapeutic agents are neurotoxic and for some of them toxic neuropathy is the dose limiting side effect. For example, the widely used anti-tumor agent cisplatin causes a predominantly large fiber sensory neuropathy which is dose limiting (usually at doses greater than 300 $mg/m^2$) (120). Vincristine, another important chemotherapeutic agent, causes a mixed neuropathy affecting sensory, motor and autonomic fibers (26). Paclitaxel (Taxol, Bristol Myers Squibb) has as its dose limiting toxicity a predominantly sensory neuropathy (109). Apart from these anti-tumor agents, numerous other medications are neurotoxic including a variety of anti-viral agents, antibiotics, and other drugs (see Table 5).

One of the earliest studies to examine the effects of neurotrophic factors on toxic neuropathy examined vinblastine neurotoxicity. When injected subcutaneously in neonatal rats, vinblastine induces massive neuronal loss in sympathetic ganglia. In 1978 Johnson demonstrated that subcutaneous administration of NGF could prevent this loss (82). He postulated that vinblastine was disrupting the retrograde transport of NGF from target tissues in the periphery through its well known ability to disrupt microtubules, and loss of trophic support resulted in cell death. Systemic administration of NGF bypassed the need for target derived growth factor by binding directly to receptors expressed on the neuronal cell bodies. Since many other neurotoxins also interfere with normal retrograde axonal transport it was reasonable to expect that this success might be generalized.

Paclitaxel also interferes with normal growth of microtubules (129). Unlike the vinca alkaloids, however, which disrupt microtubules, paclitaxel causes microtubules to aggregate excessively in disordered arrays. This too might be expected to interfere with normal axonal transport. In 1982, Peterson and Crain exposed organotypic cultures of fetal mouse dorsal root ganglia to paclitaxel and discovered that more than 95% of the cells died (130). Addition of NGF resulted in the survival of about 90%. These results suggested that NGF might be neuroprotective in this model as well. Others have demonstrated in similar *in vitro* models (with sensory and sympathetic neurons) that at lower doses, paclitaxel inhibited neurite outgrowth, and NGF administration prevented this inhibition (62, 92). Interestingly, combined treatment of PC12 cells with paclitaxel and NGF resulted in larger neurites and increased microtubule density than could be seen with the addition of NGF alone (34). This observation may be explained by the fact that NGF is able to induce microtubule organization by promoting the expression of microtubule asso-

ciated proteins (21, 45), and paclitaxel causes disordered microtubule aggregation.

We tested the ability of NGF to prevent paclitaxel induced neuropathy in a mouse model. We found that co-treatment with NGF prevented paclitaxel induced elevation of tailflick threshold (a behavioral measure of tolerance to a thermal noxious stimulus), as well as the reduction of substance P levels in dorsal root ganglia, and a mild decrease in caudal nerve action potential amplitude (7). These measures were chosen because they are particularly sensitive to small diameter fiber sensory function. In the clinical paclitaxel neuropathy, all sensory modalities are effected, however, early on patients are more likely to complain of painful dysesthesias as well as loss of pain and temperature sensation, suggesting small fiber involvement (109).

Just as NGF would be the most appropriate neurotrophic factor to use in the treatment of small fiber sensory neuropathy, NT-3 is the optimum choice for large fiber neuropathy. NT-3 has been tested in rat models of cisplatin neuropathy (56) and pyridoxine neuropathy (63 ), both of which induce a predominantly large fiber sensory neuropathy. Clinically, large fiber neuropathy results in loss of vibratory and proprioceptive sensation, leading to sensory ataxia. It is also characterized by abnormal conduction velocities on electrophysiological testing, since the large diameter fibers constitute the fastest conducting fibers. In these models, NT-3 administration successfully prevented behavioral, electrophysiological and morphological changes indicative of a large fiber sensory neuropathy.

That NGF would prevent small fiber neuropathy and NT-3, large fiber neuropathy is what might be expected on the basis of their known biology. There is evidence, however, that NGF administration may also protect against features of cisplatin neuropathy, which is primarily large fiber. The *in vitro* data is contradictory. In one study conducted by Windebank *et al*, dorsal root ganglion explant cultures were inhibited in their neurite outgrowth by the administration of cisplatin, and addition of NGF failed to prevent this inhibition (156). Konings *et al* examined a similar *in vitro* model and reported that NGF did prevent cisplatin inhibition of neurite outgrowth (92). We examined a mouse model of cisplatin neurotoxicity, and discovered that in our model NGF administration prevented behavioral, electrophysiological and biochemical changes caused by cisplatin administration (Figure 3) (8). This suggested that NGF administration might somehow support large fiber sensory neurons as well. To explain these results, we hypothesized that perhaps NGF administration might regulate

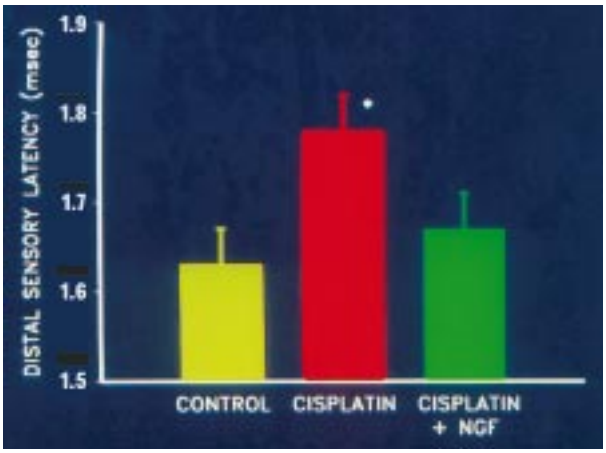



**Figure 3.** Distal sensory latency in cisplatin treated mice. Cisplatin administration to mice prolonged the distal sensory latency recorded from the caudal nerve. This translates to a reduction in nerve conduction velocity. Co-administration of NGF prevented this change. * Signifies that this group differed from the other two at $p < 0.006$ by ANOVA.

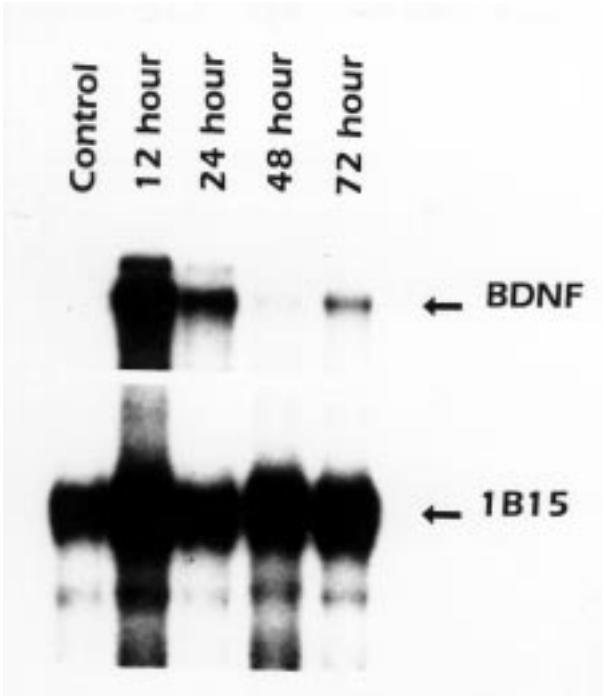



**Figure 4.** NGF administration stimulates BDNFmRNA expression in dorsal root ganglia. BDNF mRNA was maximally stimulated by 12 hours after a single injection of rhNGF. 1B15 (cyclophilin) serves as an internal standard for comparison of the amounts of mRNA loaded in each lane.

**Figure 5.** Compressive neuropathy model: Four loose ligatures are tied about the sciatic nerve resulting in hyperalgesia and trophic changes to the limb.

the expression of some other neurotrophic factor in the sensory ganglion.

To investigate this possibility, we measured levels of BDNF mRNA and NT-3 mRNA in dorsal root ganglia following administration of a single dose of recombinant human NGF (rhNGF). We did not see any changes in the expression of NT-3 mRNA, however, levels of BDNF mRNA were dramatically increased as early as 12 hours following the injection (Figure 4) (13). Double labeling in-situ studies showed that BDNF was expressed in neurons containing Trk A mRNA as well as in neurons which

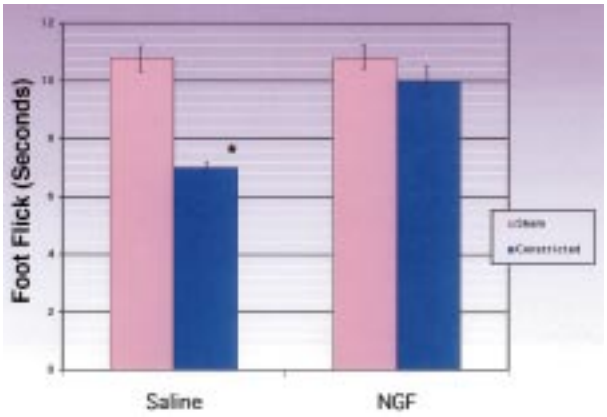



**Figure 6.** NGF administration prevents hyperalgesia associated with the chronic constriction injury. Two groups of rats were subjected to constriction of their left sciatic nerve and sham surgery on the right. The constriction injury resulted in a lowered "foot flick" threshold to a thermal noxious stimulus. Co-administration of rhNGF prevented the lowering of the threshold. The pink bar represents the sham operated side, the blue bar represents the constricted side.

did not. Both types of neurons appeared to increase their expression of BDNF mRNA, suggesting that perhaps the effect was mediated through a non-Trk A mediated mechanism. Michael *et al* have replicated the finding that NGF administration stimulates the expression of BDNF mRNA in the dorsal root ganglion, but they found that the mRNA and protein were increased selectively in Trk A expressing neurons (116). The purpose of this regulation is not known, but it has been demonstrated that BDNF may be transported anterogradely from the dorsal root ganglion into the dorsal horn of the spinal cord (116). One may speculate that BDNF might be modifying nociceptive information at the level of central connections in the dorsal horn of the spinal cord. Supporting this theory are the observations that BDNF levels are also increased with inflammation and peripheral nerve injury (31, 115). Both types of painful conditions are also associated with increased NGF expression (115). Administration of BDNF to the midbrain results in an anti-nociceptive effect in response to the tailflick, hot plate and formalin tests (147, 148), and increases the expression of substance P, β-endorphin, and neuropeptide Y in the dorsal spinal cord. These neuropeptides all participate in the modulation of pain sensation. While it is still unclear what functional role the BDNF expressed in sensory ganglion neurons has, it does suggest that systemic administration of NGF may have interesting physiological effects not predicted by its classic biology, and these will need to be considered in clinical trials.

Apart from the neurotrophins, other neurotrophic factors have been tested as potential therapies for toxic neuropathy. In an effort to address the treatment of motor neuropathies, we tested CNTF in a mouse model of vincristine neuropathy without detecting a beneficial effect (unpublished observations). Subsequently we tested IGF-I in the vincristine model and discovered that IGF-I administration could attenuate the motor and sensory neuropathy in a dose related fashion (33).

### Treatment of Compression Neuropathies and Neuropathic Pain

Compressive neuropathy is a common and often debilitating condition that may result in weakness, sensory loss, local autonomic disturbances, and very often severe neuropathic pain. Clinically this condition may include such common entities as carpal tunnel syndrome, ulnar entrapments, and nerve root compression (radiculopathy). Less common, but extremely debilitating syndromes such as reflex sympathetic dystrophy and causalgia may sometimes result from nerve compression. Bennett and Xie have established a rat model for compressive neuropathy which mimics many of the features associated with causalgia (19). To establish the model four loose ligatures are tied around the sciatic nerve, resulting in hyperalgesia and trophic changes within a few days (Figure 5).

Ren *et al* infused NGF directly on the ligated nerve via a mini osmotic pump (133). They found that both thermal and mechanical hyperalgesia normally associated with the lesion were prevented. We have replicated this observation with daily systemic injections of rhNGF beginning with the day of surgery (unpublished results). Electrophysiological and histological examination of the ligated nerve failed to show any differences between ligated nerves treated with NGF and those from animals receiving placebo injections. On behavioral testing, however, rhNGF administration resulted in a marked attenuation of both thermal and mechanical hyperalgesia compared with placebo treatment (Figure 6). The fact that NGF administration did not prevent nerve injury but did eliminate the hyperalgesic consequences of the injury raises the likelihood that it works through some other physiological mechanism. One intriguing possibility, which we are currently investigating, is that NGF might be preventing hyperalgesia at the level of the spinal cord, by stimulating the expression of BDNF which is transported anterogradely into the dorsal horn of the spinal cord. There BDNF might have an anti-nociceptive effect as described above.

| Small Fiber Sensory | Large Fiber Sensory | Autonomic |
|---|---|---|
| Burning pain | Loss of vibration sensation | Heart rate abnormalities |
| Cutaneous hyperesthasia | Loss of proprioception | Postural hypotension |
| Paresthesias | Loss of reflexes | Abnormal sweating |
| Lancinating pain | Slowed nerve conduction velocities | Gastroparesis |
| Loss of temperature | | Neuropathic diarrhea |
| Foot ulceration | | Impotence |
| Loss of visceral pain | | Retrograde ejaculation |

**Table 6.** Signs and symptoms of neuropathy.

### Diabetic Neuropathy

Neuropathy is a frequent complication of diabetes, with a prevalence of approximately 50% in patients with a 20 year history of diabetes (151). Diabetic neuropathy is a heterogeneous term that encompasses a variety of different disorders affecting the peripheral nervous system. The most common type of diabetic neuropathy is the sensorimotor and autonomic polyneuropathy which is characterized by degeneration of both small and large diameter sensory fibers early on, and only later manifests relatively moderate motor involvement. Signs and symptoms associated with degeneration of each fiber type is summarized in Table 6. Most of the symptoms that adversely affect the quality of life are caused by small fiber sensory and autonomic neuropathy. Large fiber sensory function is easier to measure quantitatively, and may also contribute towards some of the symptomatology, but in general has less impact on the patient's quality of life. Moreover, serious diabetic complications such as foot ulceration, "silent" systemic disease, and cardiac arrhythmias have a particular association with small fiber sensory and autonomic neuropathy (151). NGF, which is trophic for those particular peripheral nerve populations, was therefore an obvious choice to investigate as a potential therapy.

We examined the effects of rhNGF administration to Wistar rats made diabetic by administration of streptozocin. In this model, we found that by 13 weeks following streptozocin administration there were clear signs of small fiber sensory nerve dysfunction (10). Tailflick threshold to a thermal noxious stimulus was significantly elevated in the diabetic rats as compared with controls, suggesting loss of pain and temperature sensation (Figure 7). We also observed about a 30% reduction in levels of substance P, and CGRP in dorsal root ganglia from the diabetic animals (Figure 8). In addition to these small fiber measurements, nerve conduction velocity

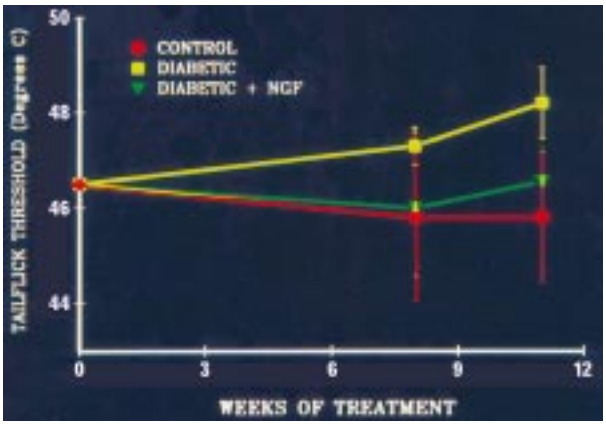



**Figure 7.** Tailflick threshold in diabetic rats. Tailflick threshold is significantly elevated by 12 weeks after streptozocin administration. Co-administration of rhNGF prevented this increase.

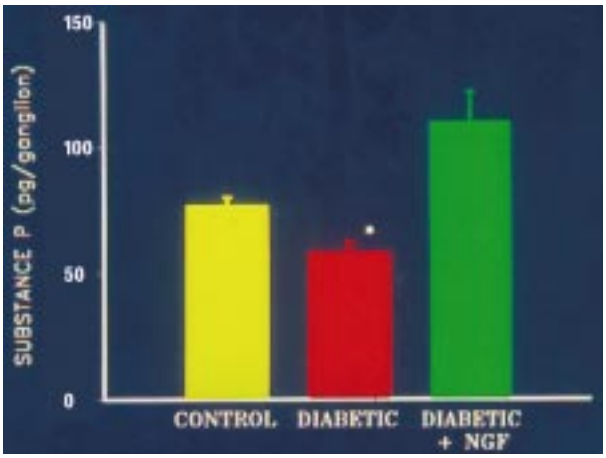



**Figure 8.** Dorsal root ganglion substance P in diabetic rats. Substance P levels were reduced by about 30% in diabetic rats, co-administration of rhNGF prevented this decrease.

recorded from the caudal nerve was reduced, suggesting some large fiber dysfunction as well. Administration of rhNGF subcutaneously three times a week throughout the 13 week period prevented the elevated tailflick threshold and the reduction in neuropeptide levels, suggesting that it might protect against small fiber sensory neuropathy. Nerve conduction velocity was only slightly improved but did not differ significantly from the placebo treated diabetic group. In a separate study, Fernyhough *et al* demonstrated that administration of rhNGF also prevented the reduction of substance P and CGRP seen in the sciatic nerve of the streptozocin rat (54).

The only other neurotrophin tested in an animal model of diabetic neuropathy was NT-3. Administration of NT-3 to streptozocin treated rats eight weeks following induction of diabetes selectively prevented a reduction in nerve conduction velocity in sensory nerves and not motor nerves (152). It is reasonable to assume that NT-3 would probably be the ideal factor to use in the treatment of large fiber sensory neuropathy seen commonly with diabetes, however, as can be seen from Table 6, most of the serious consequences of diabetic polyneuropathy are related to small fiber dysfunction, and any useful therapy will have to support this population.

Of the other neurotrophic factors, positive data has been published only with regard to IGF-I and IGF-II. Subcutaneous administration of IGF-I and IGF-II to streptozocin rats are reported to prevent the progression of hyperalgesia in this model and ameliorate the impairment of sensory nerve regeneration (78). IGF-I and IGF-II have also been shown to prevent hyperalgesia in a spontaneous rat model of non-insulin dependent diabetes (163).

### Clinical Trials

The past five years have witnessed the introduction of several neurotrophic factors to clinical trials. The earliest clinical trials to be conducted with growth factors attempted to treat amyotrophic lateral sclerosis (ALS). The first factor to be used in these trials was CNTF. In pre-clinical studies CNTF demonstrated significant trophic activity for motor neurons *in vitro* and *in vivo*. CNTF significantly improved function in several models of motor neuron disease including the wobbler mouse (119) and the progressive motor neuropathy (pmn) mouse (144). Based on success with these models two pharmaceutical companies initiated clinical trials of CNTF in the treatment of ALS. Both trials were discontinued because of toxicity which included anorexia, weight loss, fatigue, and viral-like symptoms as well as lack of efficacy (29, 118). The poor outcome of these clinical trials suggested that perhaps too little CNTF was gaining access to the motor neurons, and too much was having unwanted activities systemically. Motor neurons are protected from the systemic circulation by the blood nerve barrier, so would probably not gain ready access to CNTF administered by subcutaneous injection. It was hoped that enough would bind to terminals and be retrogradely transported to be effective. One approach around this problem would be to administer CNTF directly to the cerebral spinal fluid (CSF) space. This would increase access to diseased motor neurons and restrict systemic availability of CNTF, presumably reducing systemic toxicity. This concept was tested in one small trial where encapsulated baby ham-

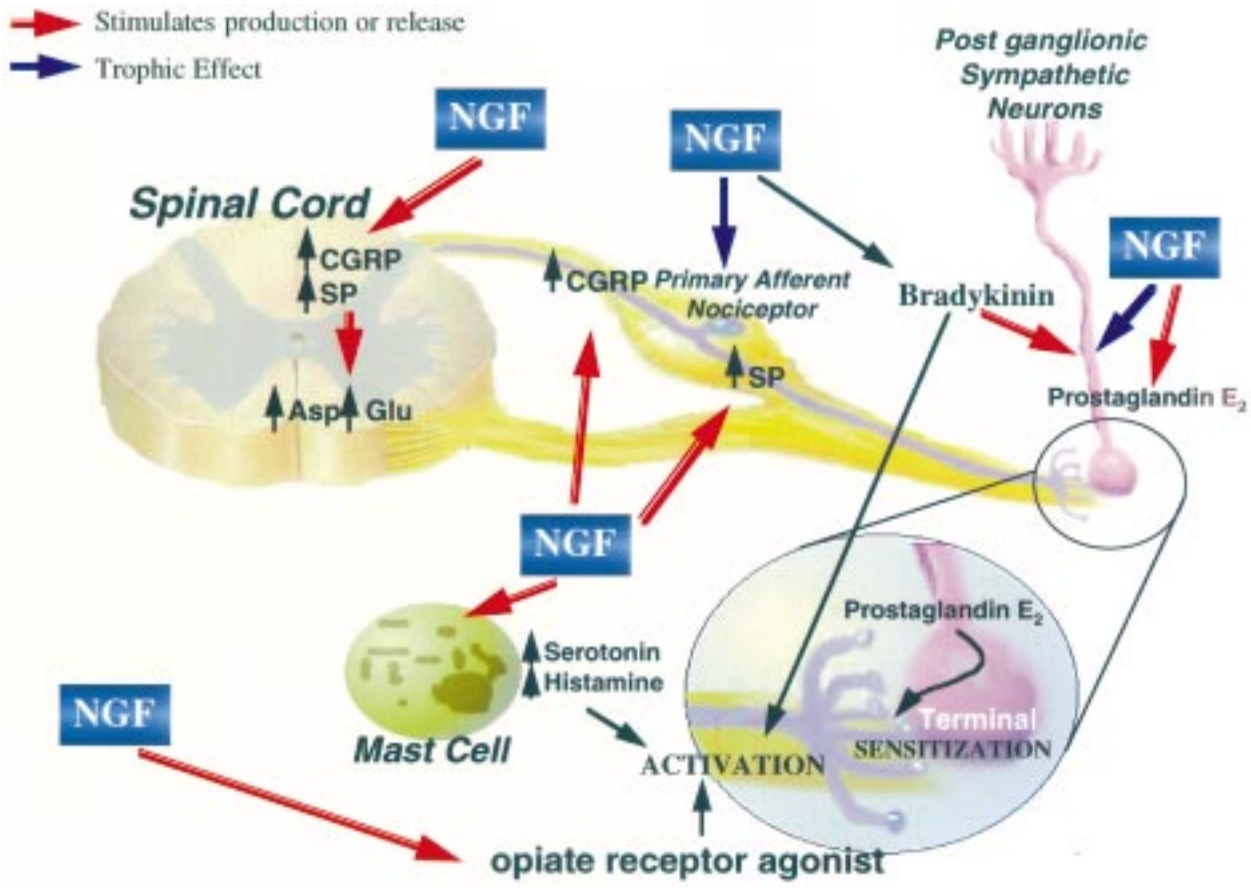



**Figure 9.** NGF induced hyperalgesia. Numerous mechanisms have been proposed to explain how administration of NGF induces hyperalgesia. NGF is trophic for nociceptive afferent and sympathetic neurons which participate in mediating pain sensation. NGF also stimulates the nociceptive neuropeptides, substance P and CGRP, induces mast cell degranulation with the release of serotonin and histamine, and potentiates the activities of bradykinin and prostaglandin E2.

ster kidney cells genetically altered to produce CNTF were implanted into the lumbar intrathecal space of six ALS patients (2). The trial was not designed to detect efficacy, however, nanogram levels of CNTF were detectable in the patient's CSF without significant systemic side effects. Whether CNTF continues to be developed for the treatment of ALS with this approach remains to be seen.

IGF-I has also been tested in two clinical trials for the treatment of ALS. A North American trial reported that administration of 0.1mg/kg/day of rhIGF-I subcutaneously, significantly delayed the progression of the disease, with treated patients living on the average 3 months longer than placebo treated patients (162). There were no major toxic side effects reported. Data from a European study failed to demonstrate a beneficial effect of IGF-I administration. Additional clinical trials will probably be necessary to clearly demonstrate a beneficial effect, however, the FDA has been requested to consider approving IGF-I for the treatment of ALS. This is still under consideration.

The only neurotrophic factor that has been tested in clinical trials for peripheral neuropathy is NGF. rhNGF has successfully completed phase I and phase II trials in the United States which have demonstrated its safety and possible efficacy in the treatment of diabetic peripheral neuropathy. The phase I trials were open label studies which revealed that rhNGF was best tolerated at doses below 1μg/kg with subcutaneous injection (11, 131). The most common side effects seen were injection site hyperalgesia, described as soreness over the injection site, and on occasion generalized myalgias or arthralgias. These will be discussed in more detail below.

The phase II clinical trial enrolled 250 patients from 15 centers across the United States (Apfel *et al*, manu-

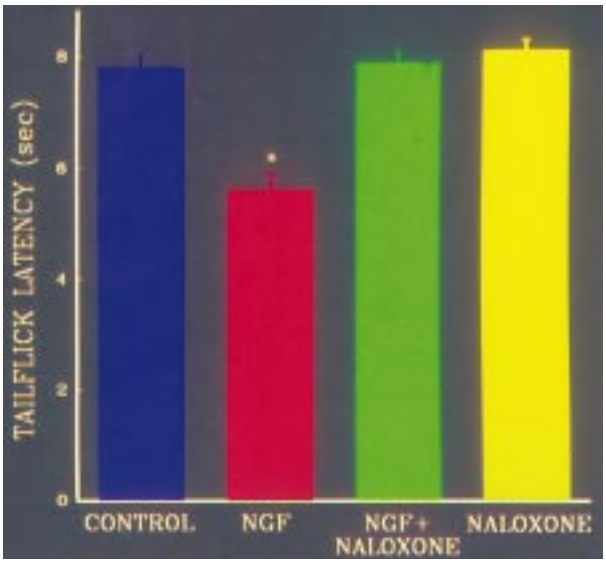



**Figure 10.** Opiate antagonists block NGF induced hyperalgesia in rats. Administration of rhNGF lowers the tailflick threshold significantly. Naloxone, an opiate receptor antagonist blocks this hyperalgesic effect.

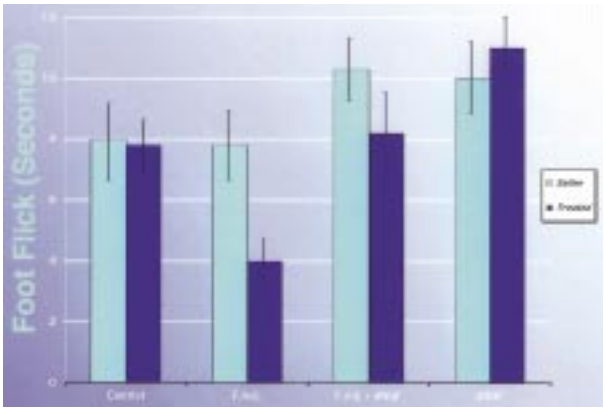



**Figure 11.** NGF antiserum prevents Freund's adjuvant induced inflammatory hyperalgesia. Light blue bars represent the foot flick latency in the saline treated foot. Dark blue bars represent footflick latency in the feet of rats given a local injection of Freund's adjuvant. aNGF stands for anti-NGF antibody.

script submitted). This trial was placebo controlled, but the high incidence of injection site hyperalgesia (>90%) may have effectively unblinded the study. Prospectively chosen multiple endpoint analyses demonstrated a significant beneficial effect of rhNGF in improving the symptoms of small fiber neuropathy over the six month treatment period. Although, the data from this study cannot be considered conclusive because of the relatively small sample size and possible unblinding, it was nevertheless considered to be encouraging by Genentech (South San Francisco, CA) which sponsored the study. Currently rhNGF is in the midst of two major phase III clinical trials for the treatment of diabetic neuropathy in the US and in Europe. These studies are investigating the efficacy of rhNGF in 1000 and 1500 subjects respectively, who are undergoing treatment for 1 year. rhNGF has also recently been tested in clinical trials for the treatment of AIDS neuropathy. The results of this trial have not yet been made public at the time that this is being written.

### Side Effects of Neurotrophic Factor Therapy

Early enthusiasm for neurotrophic factor therapy was tempered by concern regarding potential side effects of this new type of therapy. Many early concerns focused on the "growth promoting" properties of these factors. If neurotrophic factors induced sprouting of degenerating neurons, might they establish aberrant connections? Perhaps abnormal synaptic connections may even impair neurological function further. Some neurotrophic factors, most notably NGF, stimulate the expression of neuropeptides, enzymes and other metabolic proteins to levels beyond their physiological expression. Is this necessarily a good thing? Another concern relates to possible systemic side effects. Many neurotrophic factor receptors are expressed outside the nervous system, and appear to be active in a variety of different tissue types. What sort of unforseen effects might they have there? Is there a risk that large quantities of "growth factors" might serve as mitogens and be carcinogenic? Recently, for example, it was reported that high physiological serum levels of IGF-I (a mitogen for prostate epithelial cells) was positively correlated with increased risk for development of prostate cancer (30). Of course, this does not establish a causative association between IGF-I and prostate cancer, but it does increase concern regarding the exogenous administration of high concentrations of IGF-I. Will administration of neurotrophic proteins stimulate the production of auto antibodies against naturally occurring growth factors? Might we in the long run be cutting off our endogenous supply of not only the neurotrophic factor we are administering, but possibly related factors that cross react with these antibodies?

At the present time it is impossible to definitively answer any of these questions, however, there is reason to be optimistic. Numerous studies conducted *in vivo* and now with clinical trials have failed to demonstrate the reality of any of these concerns. It is clearly, however, way too early to discount them altogether. In addition, other unexpected side effects have been seen and may need to be addressed before the responsible factors

can be applied clinically. CNTF for example was discovered to induce anorexia and marked weight loss in the clinical trials. Similar effects were seen in rodent studies as well (69). CNTF induced cachexia in mice was associated with rapid catabolism of adipose tissue and muscle, decreased levels of serum glucose and triglycerides, and a loss of CD4+/CD8+ T cells. In another study it appeared that CNTF overproduction in transgenic mnd mice actually increased the rate of onset of motor neuron disease symptoms (157). While most studies support a trophic role for CNTF in protecting motor neurons, there are clearly major problems associated with CNTF administration which need to be addressed before it can be used clinically.

NGF is largely very well tolerated with no serious side effects reportedly associated with its administration. As mentioned above, however, injection site hyperalgesia has been seen in greater than 90% of the subjects receiving rhNGF in the phase II study. Other adverse events seen more commonly in the rhNGF treated groups included mildly painful conditions such as myalgias and arthralgias. The association between NGF and pain is complex, but interesting (Figure 9). In rodents, a single injection of NGF induces the rapid onset of both thermal and mechanical hyperalgesia (100). Several possible mechanistic explanations have been proposed, including serotonin release from degranulating mast cells sensitizing peripheral nociceptors (101) and activation of the bradykinin BK1 receptor (135). These mechanisms suggest a peripheral action of NGF rather than a central one which would require uptake into the nerve. This interpretation is supported by a clinical study in which NGF was administered intradermally and induced local hyperalgesia within a few hours (46). This was felt to be too short a time period for retrograde transport of NGF back to the neuronal cell body, so local effects were hypothesized to be responsible.

We found that kappa opioid receptors may also play a role in mediating NGF induced hyperalgesia. Earlier Shen and Crain had demonstrated that administration of NGF to spinal cord - dorsal root ganglion explant cultures resulted in a prolonged action potential duration as measured in the sensory neurons (146). This was identical to the prolongation of action potential duration observed with administration of low dose opioids (35). Both the NGF effect and the opioid effect could be blocked by addition of opioid receptor antagonists, and in particular by selective kappa receptor antagonists. We discovered that opioid receptor antagonists were also able to prevent NGF induced hyperalgesia in a rat model (Figure 10) (12). Although this approach has not yet been tested in humans, it does suggest a potentially simple technique for eliminating this bothersome side effect.

Interestingly, NGF production is stimulated by inflammatory cytokines (137) and is increased in inflamed tissue (155). We and others have demonstrated that administration of anti-NGF antiserum will prevent the hyperalgesia associated with Freund's adjuvant induced inflammation (Figure 11) (12,158). This suggests that NGF might play a role in mediating inflammatory pain. We have tested the effect of NGF administration on inflammatory hyperalgesia, and have not seen any exacerbation (unpublished observations). This suggests that although NGF may help mediate inflammatory hyperalgesia it should still be safe to administer to patients with inflammation.

## Conclusions

Peripheral neuropathies are likely to be the first clinical disorders to be successfully treated with neurotrophic factors. A large number of neurotrophic factors have been discovered with activity in the peripheral nervous system. Some, like GDNF and its related proteins have tremendous potential, but are still at relatively early stages of development. Others like NGF, CNTF, and IGF-I have already been tested in large scale clinical trials with varying results. Some may receive FDA approval in the next few years for specific applications in the peripheral nervous system. Others may fall by the wayside if side effects exceed efficacy. The next few years may bring us the first truly effective treatments for these common and disabling conditions.

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